

0903E60000

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[SEQ ID NO: 7]

[SEQ ID NO: 8]

[SEQ ID NO: 9]

[SEQ ID NO: 10]

[SEQ ID NO: 11]

[SEQ ID NO: 12]

[SEQ ID NO: 13]

Table 2. Sequence of PCR primers for amplifying heavy- and light-chain genes of human antibody.

(B= C/G/T; D= A/G/T; K= G/T; M= A/C; R= A/G; S= C/G; W= A/T; and Y= C/T)

a) Heavy-chain VH

5'-primers (back primers):

VH1b: 5'-ACC CCA CCA AAC CCA AAA AAA GAG ATC TGT ATG GCT
TAC CCA TAC GAT GTT CCA GAT TAC CAG GTG CAG CTG CAG GAG
TCS G-3' [SEQ ID NO: 14]

VH2b: 5'-ACC CCA CCA AAC CCA AAA AAA GAG ATC TGT ATG GCT
TAC CCA TAC GAT GTT CCA GAT TAC CAG GTA CAG CTG CAG CAG
TCA-3' [SEQ ID NO: 15]

VH3b: 5'-ACC CCA CCA AAC CCA AAA AAA GAG ATC TGT ATG GCT
TAC CCA TAC GAT GTT CCA GAT TAC CAG GTG CAG CTA CAG CAG
TGG G-3' [SEQ ID NO: 16]

VH4b: 5'-ACC CCA CCA AAC CCA AAA AAA GAG ATC TGT ATG GCT
TAC CCA TAC GAT GTT CCA GAT TAC GAG GTG CAG CTG KTG GAG
WCY-3' [SEQ ID NO: 17]

VH5b: 5'-ACC CCA CCA AAC CCA AAA AAA GAG ATC TGT ATG GCT
TAC CCA TAC GAT GTT CCA GAT TAC CAG GTC CAG CTK GTR CAG
TCT GG-3' [SEQ ID NO: 18]

VH6b: 5'-ACC CCA CCA AAC CCA AAA AAA GAG ATC TGT ATG GCT
TAC CCA TAC GAT GTT CCA GAT TAC CAG RTC ACC TTG AAG GAG
TCT G-3' [SEQ ID NO: 19]

VH7b: 5'-ACC CCA CCA AAC CCA AAA AAA GAG ATC TGT ATG GCT
TAC CCA TAC GAT GTT CCA GAT TAC CAG GTG CAG CTG GTG SAR
TCT GG-3' [SEQ ID NO: 20]

3'-primers (forward primers):

VH1f: 5'-ACT GCC TCC ACC ACC GCT GCC ACC TCC GCC AGA TCC
TCC GCC GCC TGA TCC ACC ACC GCC TGA GGA GAC RGT GAC CAG
GGT G-3' [SEQ ID NO: 21]

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Vk4b: 5'-GGC GGT GGT GGA TCA GGC GGC GGA GGA TCT GGC GGA
GGT GGC AGC GGT GGT GGA GGC AGT GAA ACG ACA CTC ACG CAG
TCT C-3' [SEQ ID NO: 41]

3'-primers (forward primers):

Vk1f: 5'-GAG ATG GTG CAC GAT GCA CAG TTG AAG TGA ACT TGC
GGG GTT TTT CAG TAT CTA CGA TTC TTT GAT TTC CAC CTT GGT
CC-3' [SEQ ID NO: 42]

Vk2f: 5'-GAG ATG GTG CAC GAT GCA CAG TTG AAG TGA ACT TGC
GGG GTT TTT CAG TAT CTA CGA TTC TTT GAT CTC CAS CTT GGT
CC-3' [SEQ ID NO: 43]

Vk3f: 5'-GAG ATG GTG CAC GAT GCA CAG TTG AAG TGA ACT TGC
GGG GTT TTT CAG TAT CTA CGA TTC TTT GAT ATC CAC TTT GGT
CC-3' [SEQ ID NO: 44]

Vk4f: 5'-GAG ATG GTG CAC GAT GCA CAG TTG AAG TGA ACT TGC
GGG GTT TTT CAG TAT CTA CGA TTC TTT AAT CTC CAG TCG TGT
CC-3' [SEQ ID NO: 45]

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[illegible]

5'-TCGA GGC GGT GGT GGA TCA GGC GGC GGA GGA TCT GGC GGA
GGT GGC AGC GGT GGT GGA GGC AGT GCG CGC TTA ATT AA-3'
[SEQ ID NO: 46]

5'-TCG ATT AAT TAA GCG CGC ACT GCC TCC ACC ACC GCT GCC
ACC TCC GCC AGA TCC TCC GCC GCC TGA TCC ACC ACC GCC-3'
[SEQ ID NO: 47]

Table 4. Sequence of PCR primers that include loxP sites for amplifying heavy- and light-chain genes of human antibody.

(B= C/G/T; D= A/G/T; K= G/T; M= A/C; R= A/G; S= C/G; W= A/T; and Y= C/T)

a) Heavy-chain VH

5'-primers (back primers):

VH1b: 5'-ACC CCA CCA AAC CCA AAA AAA GAG ATC TGT ATG GCT
TAC CCA TAC GAT GTT CCA GAT TAC CAG GTG CAG CTG CAG GAG
TCS G-3' [SEQ ID NO: 14]

VH2b: 5'-ACC CCA CCA AAC CCA AAA AAA GAG ATC TGT ATG GCT
TAC CCA TAC GAT GTT CCA GAT TAC CAG GTA CAG CTG CAG CAG
TCA-3' [SEQ ID NO: 15]

VH3b: 5'-ACC CCA CCA AAC CCA AAA AAA GAG ATC TGT ATG GCT
TAC CCA TAC GAT GTT CCA GAT TAC CAG GTG CAG CTA CAG CAG
TGG G-3' [SEQ ID NO: 16]

VH4b: 5'-ACC CCA CCA AAC CCA AAA AAA GAG ATC TGT ATG GCT
TAC CCA TAC GAT GTT CCA GAT TAC GAG GTG CAG CTG KTG GAG
WCY-3' [SEQ ID NO: 17]

VH5b: 5'-ACC CCA CCA AAC CCA AAA AAA GAG ATC TGT ATG GCT
TAC CCA TAC GAT GTT CCA GAT TAC CAG GTC CAG CTK GTR CAG
TCT GG-3' [SEQ ID NO: 18]

VH6b: 5'-ACC CCA CCA AAC CCA AAA AAA GAG ATC TGT ATG GCT
TAC CCA TAC GAT GTT CCA GAT TAC CAG RTC ACC TTG AAG GAG
TCT G-3' [SEQ ID NO: 19]

VH7b: 5'-ACC CCA CCA AAC CCA AAA AAA GAG ATC TGT ATG GCT
TAC CCA TAC GAT GTT CCA GAT TAC CAG GTG CAG CTG GTG SAR
TCT GG-3' [SEQ ID NO: 20]

3'-primers (forward primers):

VH1'f: 5'-ACT GCC TCC ACC TGA TAA CTT CGT ATA GCA TAT ATT
ATA CGA AGT TAT TGA TCC ACC ACC GCC TGA GGA GAC RGT GAC
CAG GGT G-3' [SEQ ID NO: 48]

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Vk4'b: 5'-GGC GGT GGT GGA TCA ATA ACT TCG TAT AAT ATA TGC
TAT ACG AAG TTA TCA GGT GGA GGC AGT GAA ACG ACA CTC ACG
CAG TCT C-3' [SEQ ID NO: 68]

3'-primers (forward primers):

Vk1'f: 5'-CTT CGT ATA ATG TAT GCT ATA CGA AGT TAT TTT GAT
TTC CAC CTT GGT CC-3' [SEQ ID NO: 69]

Vk2'f: 5'-CTT CGT ATA ATG TAT GCT ATA CGA AGT TAT TTT GAT
CTC CAS CTT GGT CC-3' [SEQ ID NO: 70]

Vk3'f: 5'-CTT CGT ATA ATG TAT GCT ATA CGA AGT TAT TTT GAT
ATC CAC TTT GGT CC-3' [SEQ ID NO: 71]

Vk4'f: 5'-CTT CGT ATA ATG TAT GCT ATA CGA AGT TAT TTT AAT
CTC CAG TCG TGT CC-3' [SEQ ID NO: 72]

3'-primers (forward primers) for 2nd PCR of Vλ and Vk:

Vλ/Vkf: 5'-GAG ATG GTG CAC GAT GCA CAG TTG AAG TGA ACT TGC
GGG GTT TTT CAG TAT CTA CGA TAA CTT CGT ATA ATG TAT GCT-
3' [SEQ ID NO: 73]

006290" 699E0960

SEQUENCE LISTING

<110> Zhu, Li
Hua, Shaobing

<120> HIGH THROUGHPUT GENERATION AND SCREENING OF FULLY HUMAN
ANTIBODY REPERTOIRE IN YEAST

<130> 25636-701 Seq Listing

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tgaggagacg gtgaccaggg tt 82

<210> 23
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<213> Artificial Sequence

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<210> 24
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<210> 57
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<213> Artificial Sequence

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<210> 58
<211> 86
<212> DNA
<213> Artificial Sequence

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<212> DNA
<213> Artificial Sequence

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<213> Artificial Sequence

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<213> Artificial Sequence

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 $\langle 220 \rangle$

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<212> DNA

<213> Artificial Sequence

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<213> Artificial Sequence

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<223> Description of Artificial Sequence: PCR primer

<400> 67

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agtgatattg tgmtgacbca gwctcc 86

<210> 68

<211> 85

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: PCR primer

<400> 68

ggcggtggtg gatcaataac ttcgtataat atatgctata cgaagttatc aggtggaggc 60

85

<213> Artificial Sequence

<223> Description of Artificial Sequence: PCR primer

50

<213> Artificial Sequence

<223> Description of Artificial Sequence: PCR primer

50

<213> Artificial Sequence

<223> Description of Artificial Sequence: PCR primer

50

<213> Artificial Sequence

<223> Description of Artificial Sequence: PCR primer

50

<211> 78

